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TXR#: 0056765

DATA EVALUATION RECORD[1]

STUDY TYPE: ***In vitro* Mammalian Cytogenetics** Chromosome Aberration Test in Chinese Hamster Lung (CHL) cells; OPPTS 870.5375 [' 84-2]; OECD 473.

PC CODE: 016331 **DP BARCODE:** DP410187

TEST MATERIAL (PURITY): Momfluorothrin (95.7% a.i.; Lot No. 9CM0109G)

SYNONYMS: S-1563

CITATION: Kitamoto, S. (2009); In vitro Chromosomal Aberration Test on S-1563 in Chinese Hamster Lung Cells (CHL/IU). Sumitomo Chemical Co. Ltd., Japan, Report No. RWT-0005, December 25, 2009. MRID 49020030. Unpublished

SPONSOR: Sumitomo Chemical Co., Ltd., Japan

EXECUTIVE SUMMARY: In independent mammalian cell cytogenetics assays (chromosome aberration) (MRID 449020030), cultured Chinese hamster lung (CHL/IU) cells were exposed to S-1563 (95.7% a.i.; Lot No. 9CM0109G) prepared in dimethyl sulfoxide (DMSO) at concentrations of 0, 39.1, 78.1, 156, 313, 625 or 1250 µg/mL in the absence of S9 and at 0, 40.0, 60.0, 80.0, 100, 120, 140 or 160 µg/mL in the presence of S9 mix for 6 hours in Experiment 1. For Experiment 2, cells were exposed continuously to the test material for 24 hours at 0, 4.88, 9.77, 19.5, 39.1, 78.1 or 156 µg/mL –S9 or to 0, 40.0, 60.0, 80.0, 100, 120, 140, or 160 µg/mL +S9 for 6 hours. Mitomycin C and Cyclophosphamide served as the positive controls. Two hundred metaphases per group (duplicate cultures per concentration) were scored for structural and numerical chromosome aberrations. Concentrations selected for metaphase analysis were:

Experiment 1: 0, 156, 313, and 625 µg/mL –S9 (6-hour exposure; 18-hour recovery)
0, 100, 120 and 140 µg/mL +S9 (6-hour exposure; 18-hour recovery)
Experiment 2: 0, 19.5, 39.1, and 78.1 µg/mL –S9 (continuous 24-hour exposure)
0, 100, 120 and 140 µg/mL +S9 (6-hour exposure; 18-hour recovery).

Compound precipitation was evident at ≥313 µg/mL –S9 and at ≥140 µg/mL +S9. S-1563 caused ≈50% inhibition of the growth rate at 625 µg/mL –S9 (6-hour exposure) and at 78.1 µg/mL –S9 (24-hour exposure). There was, however, no indication of a clastogenic effect at the levels selected for metaphase analysis. With S9, a growth rate of ≈38-39% was evident at 140 µg/mL (both experiments); below this concentration, growth inhibition was <50%. An increased

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incidence of cells with structural chromosome aberrations was scored at all test concentrations in both experiments and ranged from 3.5 to 9.5% at 100 to 140 µg/mL vs. 1.0 % in the concurrent controls and 1.05 ±0.82 % in the historical controls. For both experiments, the most frequently observed aberrations were chromatid breaks and chromatid exchanges. Thus, the evidence of a clastogenic response induced by S9-activated S-1563 was confirmed.

No increases in the incidence of cells with numerical aberrations were observed in any experiment with or without S9 activation. Based on these considerations, it was concluded that S-1563 induced concentration-related and reproducible increases in structural chromosome aberrations in cultured mammalian cells (Chinese hamster lung cells CHL/IU) in vitro under the conditions of the test.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for In vitro mammalian cytogenetics (chromosome aberrations) OPPTS 870.5375; OECD 473.

COMPLIANCE: Signed and dated GLP and Quality Assurance statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material S-1563

Description: Not stated

Lot/Batch: Lot No.: 9CM0109G

Purity: 95.7%

CAS#: 609346-29-4

Stability: Stable for duration of study (analytically determined: 95.6% on August 4, 2009)

Solvent: Dimethyl sulfoxide (DMSO)

2. Control materials:

Negative: Solvent (DMSO)

Positive:

Nonactivation (-S9): Mitomycin C (MMC) 0.06 µg/mL (6-hr exposure); 0.02 µg/mL (24- hrs exposure). Solvent was physiological saline

Activation (+S9) Cyclophosphamide (CP) 10 µg/mL / solvent was physiological saline

3. Activation: S9 was derived from liver homogenates of male SD rats induced with phenobarbital and 5,6-benzoflavone, and obtained commercially (Oriental Yeast Co., Ltd, Tokyo, Japan).

S9 Mix composition:

Component	**Concentration**
S9 fraction	30%
$MgCl_2$	5 mM
Glucose-6-phosphate	5 mM
NADPH	4 mM
KCl	33 mM
HEPES buffer (pH 7.2)	4 mM

4. Test cells: Chinese hamster lung cells (CHL/IU)
Cells were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan)

5. Culture medium: Eagle's minimum essential medium (MEM; Nissui Pharmaceutical Co., Ltd, Tokyo, Japan) supplemented with 10% bovine serum (Lot No.: 731681 Life Technologies, Inc., USA) in plastic dishes under a humidified atmosphere of 5% CO_2 at 37ºC.

6. Test concentrations

(a) Preliminary cytotoxicity assays With and without S9 mix: 0, 19.5, 39.1, 78.1, 156, 313, 625, 1250, 2500 and 5000 µg/mL

(b) Chromosomal aberration assay, (6 hours):	0, 39.1, 78.1, 156, 313, 625 and 1250 µg/mL in the absence of S9 mix
	0, 40.0, 60.0, 80.0, 100, 120, 140 and 160 µg/mL in the presence of S9 mix
(c) Chromosomal aberration assay, (24 hours, repeat 6 hours):	0, 4.88, 9.77, 19.5, 39.1, 78.1 and 156 µg/mL in the absence of S9 mix
	0, 40.0, 60.0, 80.0, 100, 120, 140 and 160 µg/mL in the presence of S9 mix

B. TEST PERFORMANCE

This study was conducted between 29 June and 30 September 2009.

1. **Preliminary cytotoxicity assay:** Cytotoxicity was determined prior to the cytogenetic assays. Prepared cultures were treated with the solvent or the selected test material concentrations (19.5 –5000 µg/mL +/-S9) for 6 hours or for 24 hours without S9 activation. At the end of the appropriate exposure, cell suspensions were counted and the cell growth rate was used as an indicator of cytotoxicity.

2. **Cytogenetic assay:**

a. Cell exposure time:

	Test material	Solvent control	Positive control
Non-activated:	6, 24 h	6, 24 h	6, 24 h
Activated:	6 h	6 h	6 h

b. Spindle inhibition:

Inhibition used/concentration:	Colcemid® / 0.1 µg/mL
Administration time:	1.5 hours (before cell harvest)

c. Cell harvest time after termination of treatment:

	Test material	Solvent control	Positive control
Non-activated:	24 or 0 h	24 or 0 h	24 or 0 h
Activated:	24 h	24 h	24 h

d. Details of slide preparation: At harvest, culture medium was removed and each culture was treated with 0.04% trypsin. Recovered cells were examined for growth rates and chromosome aberrations. Cell suspensions with growth rates ≥50% were centrifuged, treated with hypotonic 0.075 M KCl, fixed in methanol: acetic acid (3:1), dropped onto 2 slides and stained with 3% Giemsa.

e. Metaphase analysis

No. of cells examined per dose: 200 (100 per duplicate culture)

Scored for structural?	x	Yes		No
Scored for numerical?	x	Yes, polyploidy & endoreduplication		No
Coded prior to analysis?	x	Yes		No

3. Statistics

Statistical analysis was not used to evaluate these data.

4. Evaluation criteria

a. Assay validity:
Criteria for determination of a valid assay were not cited.

a. Positive response:
Incidence of structurally aberrant cells (excluding gaps) and that of numerically aberrant cells were classified according to the following criteria: Negative (-) <5%, Marginal (±) ≥5 – <10%, and Positive (+) ≥10%. The test compound was concluded to induce chromosomal aberrations when both of the following criteria were fulfilled: a) incidence of cells with structural aberrations excluding gaps and/or that of cells with numerical aberrations are Marginal or Positive, and b) a dose/response relationship or reproducibility is observed.

Note: Historical data for the negative and positive controls were provided by the performing laboratory.
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II. RESULTS AND DISCUSSION

A. ANALYTICAL DETERMINATIONS

The test chemical was stable in dimethyl sulfoxide (DMSO) at 0.015 and 500 mg/mL for 4 hours (at room temperature).

B. PRELIMINARY CYTOTOXICITY ASSAY

The highest concentration tested was 5000 µg/mL +/-S9. Compound precipitation was seen in the culture medium at the beginning of treatment at ≥156 µg/mL +/-S9 and at the end of treatment at ≥313 µg/mL+/-S9.

No marked inhibition of the growth rate (i.e., ≥ 50%) was seen after 6 hours of treatment with or without S9 activation. A dose-dependent growth inhibition was observed in the absence of S9 mix (24 hours treatment), ranging from 48.7% at 5000 µg/mL to 66.2% at 19.5 µg/mL. Based on these findings, 1250 µg/mL +/-S9 was selected at the starting concentration for the first experiment.

C. CYTOGENICITY AND CHROMOSOME ANALYSIS

Experiment 1

In Experiment 1, concentrations of 0, 39.1, 78.1, 156, 313, 625 and 1250 µg/mL –S9 were tested. Compound precipitation was evident at ≥313 µg/mL. Chromosome preparations from cultures treated with S-1563 at 0, 156, 313, or 625 µg/mL in the absence of S9 mix were analyzed. These data are presented in Table 1 and indicate that nonactivated S-1563 did not induce a clastogenic effect following 6-hours of treatment.

Chromosome preparations, from cultures treated with S-1563 at 0, 100, 120, or 140 µg/mL in the presence of S9 mix, were analyzed. The highest concentration tested was selected based

on a growth rate of 38.3% at 140 µg/mL; higher levels (≥ 160µg/mL) had growth rates ≤29%. The S9-activated findings from the first experiment are summarized in Table 2. As shown, increases in structurally aberrant cells were observed at 100, 120 and 140 µg/mL and ranged from 3.5 to 9.5% (excluding gaps) at 100 to 140 µg/mL, respectively, vs. 1.0% for the concurrent negative control and 1.05% ± 0.82 for the historical control. The most frequently observed aberrations were chromatid breaks and chromatid exchanges.

Experiment 2

In Experiment 2 (confirmation test) chromosome preparations from cultures treated with S-1563 at 0, 19.5, 39.1, 78.1 µg/mL –S9 (24-hour continuous exposure) and at 0, 100, 120, 140 µg/mL +S9 (6-hour exposure; 24-hour harvest time) were analyzed. In the absence of S9, the growth rate was 49.8% at 78.1 µg/mL and no increase in the incidence of structurally or numerically aberrant cells was observed at any concentration (Table 3).

Data from the confirmatory assay with S9 are presented in Table 4. As shown, the growth rate at 140 µg/mL was 39.9% and >50% growth rates were recorded for the other levels. In agreement with the data from the first experiment, increases in structural chromosome aberrations (5.0, 7.5 and 9.0% vs. 1.5% in solvent control) were observed at 100, 120 and 140 µg/mL, respectively. Similarly, the most frequently observed aberrations were chromatid breaks and chromatid exchanges.

Numerical aberrations were not appreciably different from the solvent control cultures in either of the two experiments. Both positive control compounds (MMC and CP), induced the expected marked increases in the incidence of structural chromosome aberrations in both experiments.

Table 1 Cytogenetics In-Vitro-Test: Chromosomal Analysis (Experiment 1, 6-hour treatment /18-hour recovery) Chinese Hamster Lung Cells Exposed to S-1563

		Control DMSO solvent only	Positive control (MMC 0.06 µg/mL)	S-1563		
				156 µg/mL	313 µg/mL	625 µg/mL
Metabolic activation		**-S9**	**-S9**	**-S9**	**-S9**	**-S9**
Cytotoxicity (% growth rate compared with solvent control)		100	85.5	65.1	54.8	52.7
Structural aberrations	Chromatid/chromosome gaps	0	5	0	0	0
	Chromatid breaks	2	30	1	2	3
	Chromatid exchanges	0	9	0	0	1
	Chromosome breaks	0	0	0	0	0
	Chromosome exchanges	0	0	0	0	0
	Cells with more than 9 aberrations	0	1	0	0	0
% Cells with aberrations	**+G**	1.0	19.0	0.5	1.0	2.0
	-G	1.0	17.0	0.5	1.0	2.0
% Polyploidy and endoreduplicated cells		0.0	0.0	2.5	0.5	0.5
Comments		Precipitation of test material was observed at the start of the treatment (156 µg/mL) & at the start and end of treatment at and above 313 µg/mL.				

+G: aberrations including gaps; -G: aberrations excluding gaps
N.B. Data were extracted from the study report, Table 2, p.25, MRID 49020030.

Table 2: Cytogenetics *In-Vitro*-Test: Chromosomal Analysis (Experiment 1, 6-hour treatment /18-hour recovery) Chinese Hamster Lung Cells Exposed to S-1563

		Control DMSO solvent only	Positive control (CP 10.0 µg/mL)	S-1563		
				100 µg/mL	120 µg/mL	140 µg/mL
Metabolic activation		**+S9**	**+S9**	**+S9**	**+S9**	**+S9**
Cytotoxicity (% growth rate compared with solvent control)		100	49.9	63.3	52.2	38.3
Structural aberrations	Chromatid/chromosome gaps	1	17	1	5	4
	Chromatid breaks	0	70	6	10	8
	Chromatid exchanges	1	80	7	13	16
	Chromosome breaks	0	0	0	0	0
	Chromosome exchanges	1	0	0	0	0
	Cells with more than 9 aberrations	0	0	0	0	0
% Cells with aberrations	**+G**	1.5	52.0	4.0	10.0	11.0
	-G	1.0	48.5	3.5	8.5	9.5
% Polyploidy and endoreduplicated cells		0.0	0.5	4.5	2.0	3.5
Comments		Precipitation of test material was observed at the start of the treatment at 140 µg/mL. The structural aberration count recorded at 120 and 140 µg/mL were both judged to be marginal positive results (±). Positive control chemical CP showed clear positive results (+).				

+G: aberrations including gaps; -G: aberrations excluding gaps

N.B. Data were extracted from the study report, Table 3, p.26, MRID 49020030.

Table 3: Cytogenetics In-Vitro-Test: Chromosomal Analysis (Experiment 2, 24-hour continuous treatment) Chinese Hamster Lung Cells Exposed to S-1563

		Control DMSO solvent only	Positive control (MMC 0.02 µg/mL)	S-1563		
				19.5 µg/mL	39.1 µg/mL	78.1 µg/mL
Metabolic activation		**-S9**	**-S9**	**-S9**	**-S9**	**-S9**
Cytotoxicity (% growth rate compared with solvent control)		100	84.3	73.9	58.5	49.8
Structural aberrations	Chromatid/chromosome gaps	1	5	0	0	1
	Chromatid breaks	2	23	0	1	1
	Chromatid exchanges	1	14	0	0	0
	Chromosome breaks	0	0	0	0	0
	Chromosome exchanges	0	0	0	0	0
	Cells with more than 9 aberrations	0	1	0	0	0
% Cells with aberrations	**+G**	2.0	18.0	0.0	0.5	1.0
	-G	1.5	16.5	0.0	0.5	0.5
% Polyploidy and endoreduplicated cells		0.0	0.0	0.0	0.5	0.0
Comments						

+G: aberrations including gaps; -G: aberrations excluding gaps

N.B. Data were extracted from the study report, Table 4, p.27, MRID 49020030.

Table 4: Cytogenetics *In-Vitro*-Test: Chromosomal Analysis (Experiment 2, 6-hour treatment /18 hours recovery) Chinese Hamster Lung Cells Exposed to S-1563

		Control DMSO solvent only	Positive control (CP 10.0 µg/mL)	S-1563		
				100 µg/mL	120 µg/mL	140 µg/mL
Metabolic activation		**+S9**	**+S9**	**+S9**	**+S9**	**+S9**
Cytotoxicity (% growth rate compared with solvent control)		100	54.9	67.0	59.0	39.9
Structural aberrations	Chromatid/chromosome gaps	0	15	2	3	3
	Chromatid breaks	1	44	7	12	7
	Chromatid exchanges	2	56	5	8	15
	Chromosome breaks	0	0	0	0	0
	Chromosome exchanges	0	0	0	0	0
	Cells with more than 9 aberrations	0	1	0	0	0
% Cells with aberrations	**+G**	1.5	38.5	6.0	8.5	10.0
	-G	1.5	33.5	5.0	7.5	9.0
% Polyploidy and endoreduplicated cells		0.5	0.0	2.5	1.5	1.5
Comments		Precipitation of test material was observed at the start of the treatment at 140 µg/mL. The structural aberration count recorded at 100, 120 and 140 µg/mL (without gaps) was judged to be marginal positive results (±). Positive control chemical CP showed clear positive results (+).				

+G: aberrations including gaps; -G: aberrations excluding gaps
N.B. Data were extracted from the study report, Table 5, p.28, MRID 49020030.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS: The investigators concluded that S-1563 has a marginal potential to induce chromosomal aberrations in cultured mammalian cells (Chinese hamster lung cells CHL/IU) *in vitro* under the conditions of the test.

B. REVIEWERS' COMMENTS: In general, the reviewers agree with the investigator's conclusions. S-1563 was tested to insoluble and cytotoxic concentrations (≤50% reduction in cell survival) for either a 6-hour exposure or a continuous 24-hour exposure without S9 activation but failed to induce either a numerical or structural change in treated Chinese hamster lung cells. With S9 activation, however, S-1563 induced a reproducible and concentration-related increase in structural chromosomes aberrations. These findings were confirmed in an independently performed repeat assay. We conclude, therefore, that S-1563 is clastogenic in this test system in an acceptable/guideline *in vitro* cytogenetic test.

C. STUDY DEFICIENCIES: None.